

The Behavior of the Cercariae of
Bucephalus elegans with Special Reference to the Effect of Light and Temperature

GERRIT BEVELANDER

DISSERTATION SUBMITTED TO THE BOARD OF STUDIES, THE JOHNS HOPKINS
UNIVERSITY IN CONFORMITY WITH THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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RESPONSE TO LIGHT IN THE CERCARIAE OF BUCEPHALUS ELEGANS¹

(Six figures)

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NUMEROUS investigators have studied the problems concerning responses to light in organisms such as orientation, aggregation, sensitization, relative effect of wave lengths and intensities, and the effect of sudden change of intensity.

Reactions which are dependent on sudden change of intensity have been found to occur in many organisms (Mast, 1911, pp. 246-51). Some organisms respond to increase, others to decrease, and still others to both increase and decrease, in light intensity.

If the change in intensity to which these organisms are subjected does not take place rapidly, a response usually does not occur. It is evident therefore that the reaction is dependent upon the rate of change in light intensity. These reactions are now generally known as "shock reactions." They are characterized by the fact that the energy necessary to induce a reaction must be increased or decreased at a certain rate. The minimum amount of change in energy necessary to induce a response is known as the threshold.

In many organisms it is not necessary to expose the receptors continuously in order to induce a response. There is a period during which they must be exposed to light, followed by a period during which exposure is not necessary. If, for example, photosensitive seedlings are exposed to a horizontal beam of light for a short time and then placed in darkness, the seedlings will bend in the same direction that they would have bent had they remained in the light continuously. The time during which the stimulating agent must act in order to induce a response has been designated stimulation

¹ Contribution from the Zoölogical Laboratory of the Johns Hopkins University, and the Mt. Desert Island Biological Laboratory. The writer is very much indebted to Professor H. T. Folger, who suggested the problem and directed some of the work, and to Professor S. O. Mast, for invaluable suggestions and criticism made during the course of the experimental observations and the preparation of the manuscript.

period, exposure period, sensitization period, and presentation time; and the period during which exposure is not necessary, latent period. The sum of these two periods is known as the reaction-time. The time which elapses from the application of the stimulus until the organism resumes its former physiological state has been designated the recovery period.

The relations which exist between the stimulating agent and the various phases of response have been investigated in many organisms. The first attempt at a quantitative analysis of the response to light was made independently by Blaauw (1908 and 1909) and Froeschel (1908). These authors exposed the plumules of seedlings of *Avena* in a horizontal beam of light and ascertained in different intensities the duration of the illumination required for 50 per cent of them to bend. They maintain that the time (t) varies inversely with the intensity (i), that the product of the intensity and the time is constant, and that their results are therefore in accord with the Bunsen-Roscoe law. Ewald (1914) maintains that in *Daphnia* the amount of light energy required to evoke a response is constant over a large range of intensities. Hecht (1918, 1919a) maintains that it holds for *Ciona* and *Mya*, and Obreshkove (1921) concludes that it holds for the tadpoles of *Rana* under certain conditions.

Arisz (1911) contends that in *Avena* there is no definite threshold, that is, that, no matter how small the amount of energy is, it acts on the plant and is expressed by an effect, and that it is impossible to ascertain when a response begins. Folger (1925) maintains that in *Amoeba proteus* the amount of light energy required to induce a response varies with the intensity. Pieron (1926) repeated the work of Hecht on *Mya* and concluded that higher intensities are more efficient in inducing a response than lower intensities, i.e., that the Bunsen-Roscoe law does not hold. Folger (1926) also repeated the work of Hecht and his results confirm Pieron's contentions. Burkhardt (1926) repeated the observations of Blaauw and Froeschel on the seedlings of *Avena*, and the results which he obtained do not confirm their conclusions that the Bunsen-Roscoe law holds.

From the preceding account it is evident that in regard to the relation between intensity of illumination and the stimulating effect of a given amount of light there are divergent views. Blaauw, Froe-

schel, Loeb, Hecht, and others maintain that, if the product of the intensity multiplied by the time is constant, the response is constant, no matter how the two factors vary, i.e., that the Bunsen-Roscoe law holds. Folger, Pieron, Burkhardt, and others maintain that the stimulating efficiency of light varies with the intensity, i.e., that the Bunsen-Roscoe law does not hold.

Mast (1923, p. 179) in a discussion concerning this law concludes that "there are no reactions to light which are not in accord with the Bunsen-Roscoe law in certain respects and that there are none which are in accord with this law in all respects," and that "the statement so frequently made without qualification that this or that reaction is in accord with the Bunsen-Roscoe law is meaningless."

The present investigation concerns the behavior of the cercariae of *Bucephalus elegans* with special reference to the quantitative relation between stimulation and response to light.

MATERIAL

The cercariae of *Bucephalus elegans* are immature trematodes found in the gonad region of the fresh-water mussel *Eurynia iris* (Lea). The mussels occur in shallow beds in the Huron River, near Ann Arbor, Michigan. During the course of this investigation several hundred specimens were collected in this region.

Only a small proportion of the mussels collected were parasitized. They were identified as follows: all the specimens were put into finger bowls, one in each, and kept at a temperature of slightly more than 20°. At this temperature infected mussels readily shed their cercariae. Frequent examinations were made over a period of several days to ascertain which specimens were parasitized.

Following the period of isolation, the parasitized specimens were put into cylindrical battery jars, one in each jar, which had been previously supplied with tap water and a rich supply of green organisms. This "set up" was found to be very efficacious, for mussels were kept alive and in good condition for more than eight months.

A heavily parasitized mussel, when brought into the laboratory at ordinary room temperature, often sheds as many as one hundred cercariae within 24 hours. This was usually undesirable, and in order to obviate this high rate of shedding, the jars containing the mussels

were kept at a temperature below 15° . This simple procedure had the desired effect of reducing the shedding rate to practically zero. By keeping the mussels at a low temperature, it was possible to conserve the supply of cercariae over a period of several months. Whenever cercariae were needed for experiment, one of the jars containing a parasitized mussel was brought into the laboratory and as soon as the contents of the jar reached a temperature of 20° cercariae usually began to emerge. It appears that the most important factor in shedding parasites by this mussel is the temperature.

LOCOMOTION

The cercariae of *Bucephalus elegans* are expelled through the exhalant siphon of the mussel and immediately following liberation they start swimming toward the surface of the water. The most striking feature in their locomotion is the enormous contraction and expansion of the tail-furcae (Fig. 1, *a, b, c*). The body undergoes an undulating movement, but in comparison with that of the furcae, this movement is negligible. In swimming, the body hangs downward, with the furcae extended outward and upward in the form of a sigmoid curve. By means of extension and contraction of the furcae, the cercariae move slowly and gracefully through the water, seemingly in any direction. In swimming, the long axis of the body usually assumes a vertical position.

METHODS

The experiments on the reactions to light in the cercariae of *Bucephalus elegans* were made in a basement dark room; observations were made both with and without the aid of magnification. The source of light used was a 115-volt, 1,000-watt stereopticon lamp, mounted in a light tight shaft which was constantly ventilated so as to avoid excessive rise in temperature. The lamp was connected with a direct-current lighting system and maintained at 110 volts by means of variable resistance. A horizontal beam of light from an aperture 3 cm. in diameter was transmitted directly to a vertical microscope stage on which an observation aquarium was held by means of a mechanical stage (Fig. 2). The observation aquarium used in these experiments was constructed by fastening, with De

Khotinsky's cement, modified glass depression cells to microscope slides. Part of the cell was cut away to make an opening for the introduction of the cercariae. The aquarium was U-shaped inside and measured $20 \times 15 \times 3$ mm. (Fig. 3). Different intensities were secured by moving the microscope stand closer to, or farther from the light.

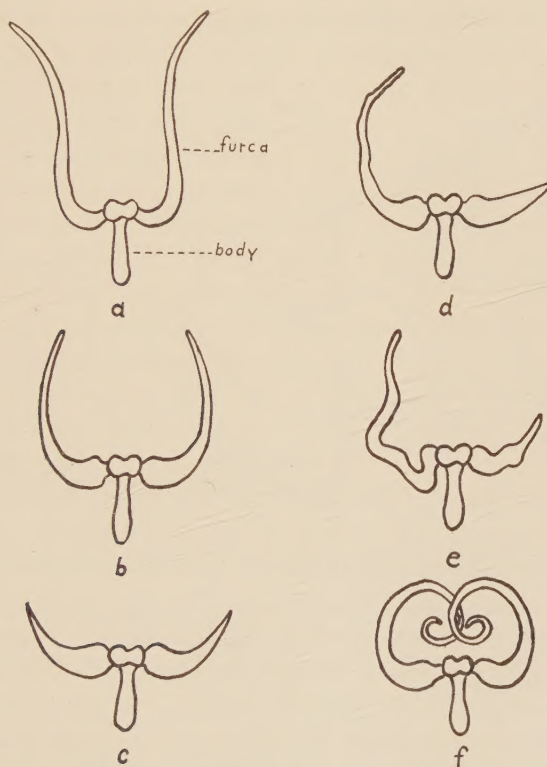


FIG. 1.—Outlines illustrating swimming movements of cercariae: *a, b, c*, normal movements; *d, e, f*, response to rapid increase in illumination.

A permanent scale in centimeters made by measuring the distance from the center of the lamp to the center of the observation aquarium on the microscope stage was marked off on a rack which supported the microscope stand. This made it possible to obtain any desired intensity rapidly and accurately. The light was turned on and off by means of a photographic shutter mounted in front of the

aperture in the wall of the ventilating shaft. The light passed through a cell 3 cm. thick containing distilled water interposed between the shutter and the lamp (Fig. 2).

RESPONSE TO RAPID INCREASE IN ILLUMINATION

When subjected to sudden increase in illumination cercariae usually respond with a slight shuddering movement, followed by an

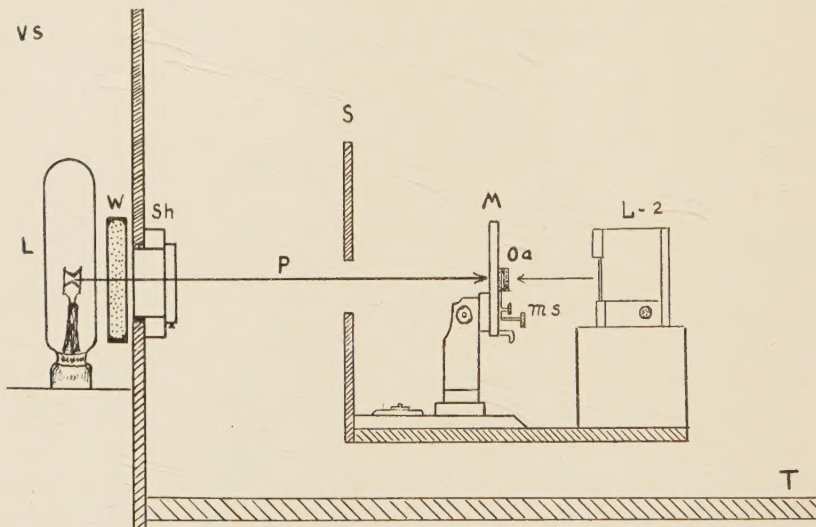


FIG. 2.—Diagram of apparatus used in studying the reactions of cercariae to light. *L*, 1,000 watt lamp, *L*₂, weak ruby lamp; *M*, microscope stand; *Oa*, observation aquarium; *P*, path of light; *S*, screen; *sh*, shutter; *ms*, mechanical stage; *T*, table; *vs*, ventilating shaft; *W*, distilled water.

inward coiling of the furcae (Fig. 1, *d e, f*), after which they slowly sink to the bottom of the aquarium. This reaction occurs only when the intensity is rapidly increased. If the intensity is gradually increased it does not occur. The cercariae recover either in light or darkness; recovery in darkness requires about one minute. In light it takes considerably longer. Recovery takes place as follows: at first the furcae slowly uncoil, then they undergo a shuddering movement, after which erratic expansion and contraction occur, and finally they swim normally again (Fig. 1, *f-a*).

Under favorable conditions the cessation of movement following

rapid increase in illumination is very marked and can be clearly seen. This made it possible to measure fairly accurately the time occupied by the different phases of response described below.

The observations on response to rapid increase in illumination were made as follows: a cercaria to be used for experiment was taken with a pipette, put into an observation aquarium in the dark room, and kept there for an hour or more to insure complete adaptation to the conditions of the room. A weak ruby light which did not appreciably affect the cercariae was now turned on, after which the aquarium containing the adapted cercaria was fastened to the mechanical stage on the microscope, and the stage placed at the desired distance from the lamp.² Then by opening the shutter the strong light from the projection lamp was suddenly flashed on the cercaria and left until a reaction occurred, after which the shutter was closed and left for 3 minutes and then opened again. The reaction-time was measured with a split-second stop watch. The number of times a specimen responded in succession varied somewhat, depending on its physiological condition. Some of the cercariae ceased to react after one or two exposures, others responded more than sixty times at 3-minute intervals with a maximum variation of only 5 per cent in the reaction-time.

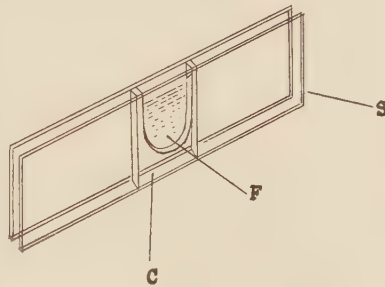


FIG. 3.—Sketch showing construction of observation aquarium. C, cell; F, fluid, S, microscope slide.

RELATION BETWEEN REACTION-TIME AND LUMINOUS INTENSITY

Table I contains the reaction-time obtained as described above for several specimens each subjected to light of diverse intensities. By referring to this table it will be seen that in every individual tested the reaction-time varied inversely with the luminous intensity,

² In measuring the reaction-time to light in the cercariae of *Bucephalus elegans*, precaution was always taken to illuminate the entire body and furcae of the specimens from the side; thus any possible effect due to differential sensitivity in the specimen was eliminated.

but that there was some individual variation; the reaction-time of different individuals in the same intensity varies from 8 to 12 seconds in the low intensities.

By referring to Figure 4 it will be seen that if the total average of the reaction-time obtained for all the individuals treated is plotted against the luminous intensity, a curve is produced which simulates a hyperbola. The relation between reaction-time and luminous intensity for the cercariae of *Bucephalus elegans* is in fairly close agree-

TABLE I
RELATION BETWEEN REACTION-TIME AND LUMINOUS INTENSITY

INTENSITY M.C.	AVERAGE REACTION-TIME (SECONDS) 3-5 READINGS OF INDIVIDUALS a-h								TOTAL AVER- AGE
	a	b	c	d	e	f	g	h	
16,800.....	3.5	2.2	4.5	2.6	3.4	4.0	3.2	2.6	2.9
12,640.....	4.1	2.6	4.6	3.7	3.9		3.4	3.7	3.8
9,480.....	4.9	4.8	5.3	4.5	5.5	5.3	4.8	5.5	5.0
6,320.....	5.2	6.0	5.7	7.3	7.1	5.4	7.0	7.5	6.4
4,740.....	7.5		8.3			8.4			8.0
3,160.....	8.4	11.7	10.3	13.5	8.6		12.7	12.6	11.1
1,580.....	13.1	23.0	13.6	24.9	13.3	11.5	23.7	21.7	18.1

ment with that obtained by others for other organisms, e.g., that obtained by Blaauw for *Avena*, by Hecht for *Ciona* and *Mya*, and by Folger for *Amoeba*.

RELATION BETWEEN STIMULATION PERIOD, LATENT PERIOD, AND LUMINOUS INTENSITY

As previously stated it has been observed that the reaction-time is made up of two components: the stimulation period, the time during which it is necessary to expose the receptors; and the latent period, the time during which it is not necessary to expose the receptors in order to elicit a response.

The stimulation period was ascertained as follows: the reaction-time for a selected cercaria was ascertained for a given luminous intensity, then the cercaria was exposed successively for shorter and shorter periods of time with an interval of 3 minutes between the successive exposures, until no response occurred. The minimum

time during which the light must act to produce a response was thus ascertained; this is the stimulation period. This procedure was then repeated with 15 other individuals at diverse intensities. The results obtained are presented in Table II and Figure 5.

Examination of Table II shows that, in the specimens tested, the stimulation period varied inversely with the luminous intensity,

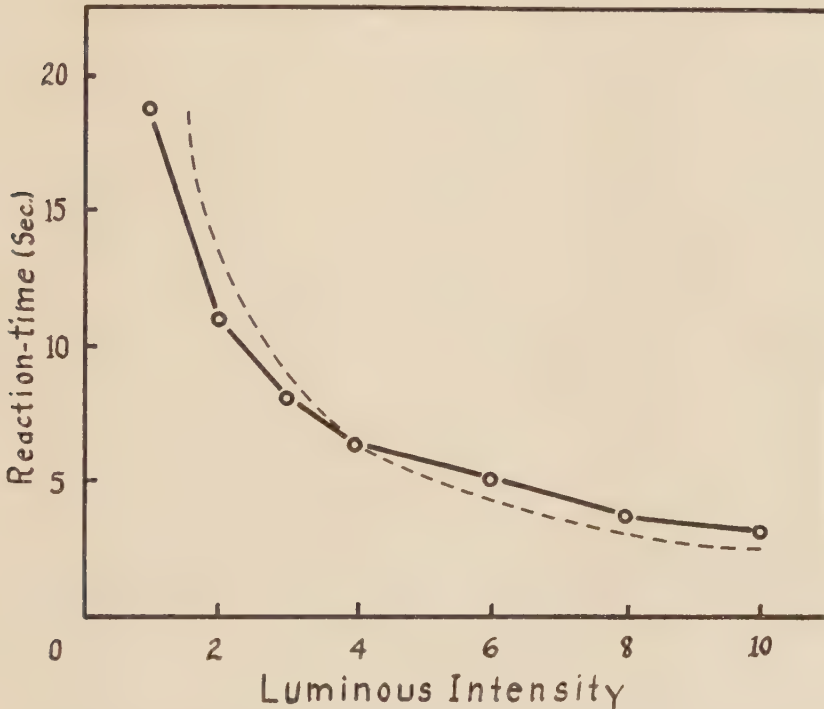


FIG. 4.—Relation between reaction-time and luminous intensity. Total average reaction-time for 8 specimens, 5 readings made on each specimen.

i.e., the more intense the light, the shorter the exposure required to obtain a response. It also shows that the latent period varied inversely with luminous intensity. It shows moreover that as the intensity decreased the energy required to induce a response rose to a maximum at 3,160 m.c. and then fell. This shows that the Bunsen-Roscoe law does not hold for the cercariae of *Bucephalus elegans* under the conditions of the experiment.

EFFECT OF TEMPERATURE ON PHOTIC REACTION-TIME

Several investigators maintain that temperature has an effect on photic response. De Vries (1913) maintains that in the seedlings of *Avena* the reaction-time depends upon the temperature. Hecht (1919c) comes to the same conclusion regarding *Mya*, and Cole (1922) contends that photic reaction-time in *Necturus* varies with the temperature. Crozier and Federighi (1924) maintain that in-

TABLE II

THE RELATION BETWEEN LUMINOUS INTENSITY, STIMULATION PERIOD,
LATENT PERIOD, AND STIMULATION PERIOD TIMES
LUMINOUS INTENSITY

Average in seconds of the results obtained by making 5-8 readings on each of 16 specimens. S.P., stimulation period; L.P., latent period; R.T., reaction-time; (it), intensity times stimulation period.

Intensity M.C.	S.P.	L.P.	R.T.	(it)
15,800.....	.8	3.2	4.0	12,640
12,640.....	1.1	3.1	4.4	13,904
8,480.....	1.9	3.7	5.6	16,112
6,320.....	2.9	3.8	6.7	18,328
3,160.....	5.6	4.7	10.3	18,696
1,580.....	6.7	5.8	12.5	10,586

crease in temperature accentuates photic circus movements in *Limax*. These investigators agree in that all maintain that increase in temperature results in decrease in the time required for various responses to light.

MATERIAL AND METHODS

The apparatus used for regulating temperature consisted of a copper water bath having a circular opening $\frac{3}{4}$ inch in diameter in one side to admit light, a certified thermometer, and five 19-liter reservoirs fitted with glass and rubber tubing. By regulating the rate of flow of water through the water bath any desired temperature could quickly and accurately be obtained and maintained as long as desired.

The observations were made as follows: an observation aquarium containing a dark adapted cercaria was fastened with metal clips to the side of the water bath so that the center of the aquarium coincided with the center of the aperture. The water bath was so placed

that the light from the lamp passed directly through the center of the aquarium. The cercaria was then exposed to the light from the lamp until a reaction occurred, after which the shutter was closed and the cercaria left in darkness. During this interval the reaction-time was recorded, then the process was repeated five times with the same individual in the same intensity after which it was repeated with the

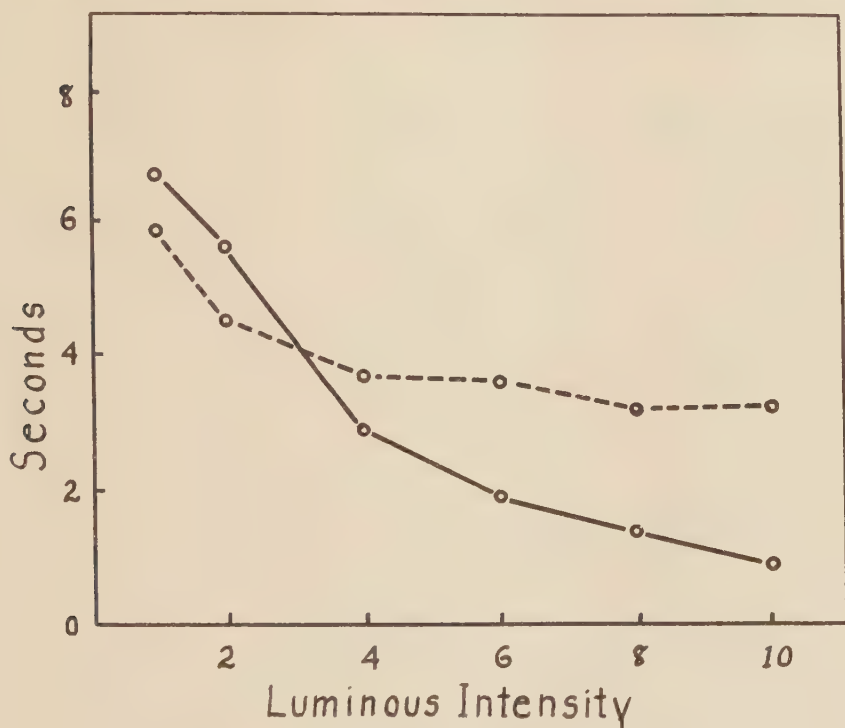


FIG. 5.—Relation between luminous intensity, stimulation period, and latent period. Curves represent the average results obtained in making 5–8 observations on each of 16 specimens. Solid line, stimulation period; broken line, latent period.

same individual in different temperatures allowing 10 minutes for adaptation at successive temperatures. This whole process was then repeated with each of 10 specimens.

RESULTS

The results obtained are given in Tables III and IV, and in Figure 6. Table III shows that the averages of the reaction-time for 5 indi-

viduals in 1,580 m.c. at temperatures 15°, 20°, and 25° were 15.1, 13.0, and 10.9 seconds, respectively, and in 9,480 m.c., 4.5, 3.9, and 2.7 seconds respectively. Table IV shows that the averages of the

TABLE III

RELATION BETWEEN TEMPERATURE AND PHOTIC REACTION-TIME
Each figure is the average of 5 readings.

DESIGNATION OF INDIVIDUALS	AVERAGE REACTION-TIME (SECONDS)					
	1,580 M.C.			9,480 M.C.		
	15°	20°	25°	15°	20°	25°
1.....	15.8	13.4	11.1	4.8	4.1	2.8
2.....	15.6	12.8	10.7	4.4	3.6	2.9
3.....	14.6	13.0	10.8	4.0	3.5	2.6
4.....	14.7	13.3	11.0	4.5	3.8	2.5
5.....	15.1	12.7	10.8	4.1	3.5	3.1
Total average.....	15.1	13.0	10.9	4.5	3.9	2.7

TABLE IV

RELATION BETWEEN TEMPERATURE AND PHOTIC REACTION-TIME IN 4,000 M.C.
Each figure is the average of 5 readings.

DESIGNATION OF INDIVIDUALS	AVERAGE REACTION-TIME (SECONDS) IN 4,000 M.C.				
	10°	12.5°	15°	20°	25°
1.....	6.7	4.8	4.6	3.6	2.8
2.....	6.7	5.6	4.4	4.0	2.7
3.....	6.6	4.8	4.2	3.8	3.1
4.....	6.8	4.9	4.2	3.5	2.6
5.....	6.5	5.0	4.4	3.6	2.3
Total average.....	6.6	5.0	4.3	3.6	2.7

reaction-time for 5 other individuals in 4,000 m.c. at temperatures 10°, 12.5°, 15°, 20°, and 25° were 6.6, 5.0, 4.3, 3.6, and 2.7 seconds, respectively. This clearly demonstrates that increase in temperature caused a decrease in photic reaction-time over a wide range of intensities.

The relation between photic reaction-time and temperature is more clearly shown in Figure 6. This figure definitely indicates that the photic reaction-time varies inversely with the temperature, and

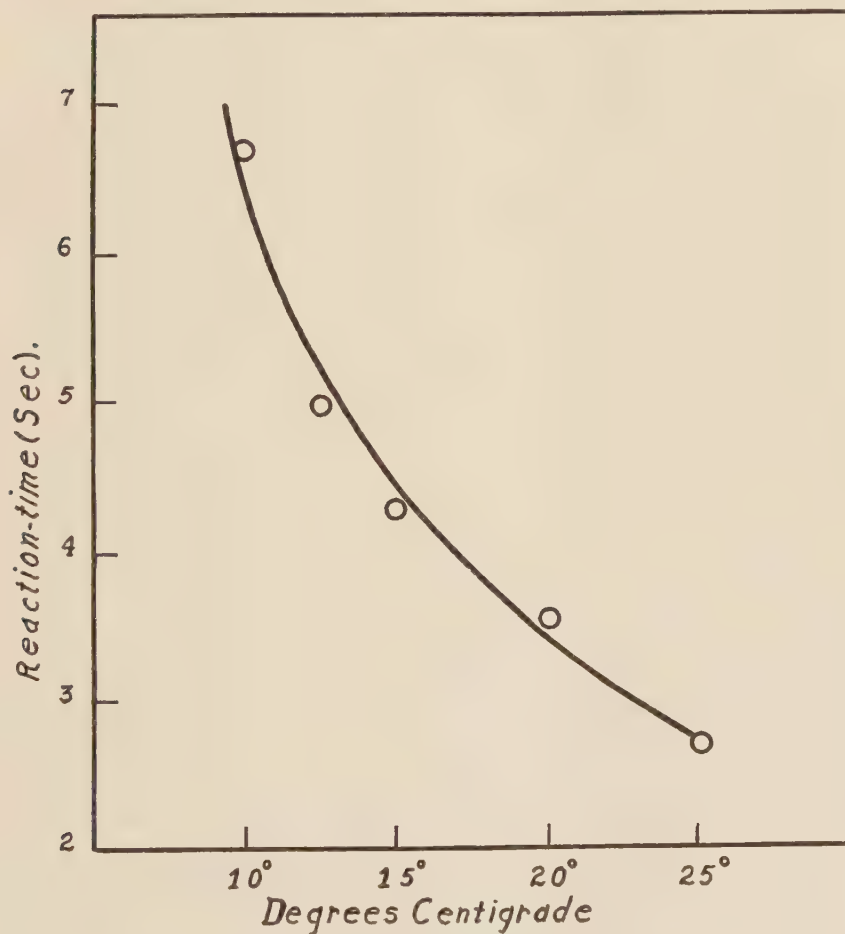


FIG. 6.—Relation between temperature and photic reaction-time in 4,000 m.c. Circles, experimental points; curve, a hyperbola (abscissae $\times$ ordinate = 67).

that the relation between reaction-time and temperature follows the equation $xy = K$ when x equals the reaction-time, y , the temperature, and K , 67.

DISCUSSION

It has been held by some investigators that certain responses to light in organisms are correlated with photochemical reactions. Mast (1907, p. 159) postulated a reversible photochemical reaction to account for certain responses to light in *Volvox*. He assumes (1) that *Volvox* contains substances X and Y , the chemical reaction between which is regulated by the intensity of the light; (2) that a sub-optimum intensity or darkness favors the formation of substances represented by X and a supra-optimum, those represented by Y ; and (3) that the colonies are neutral in reaction when there are Y substances in one member of the equation and X in the other; positive when one member contains $X+$ substances and the other ($Y-$), and negative when one contains ($X-$) and the other ($Y+$).

He later (1927) modified this hypothesis by postulating two additional substances M and N which are non-photochemical but react with X and Y in such a way as to account for the effect of adaptation on the response.

Hecht (1918, 1919a, b, 1921), to account for the response to light in *Ciona* and *Mya*, postulated a reversible photochemical reaction like that postulated by Mast in his earlier work. He assumed that a substance S is changed under the influence of light to substances P and A , that a certain amount of S must be broken down in order to induce a response, that the stimulation period is the time necessary to convert the required amount of S to P and A , and that the latent period is the time during which other processes occur which are not dependent upon light.

The response to light of cercariae studied are fundamentally the same as those observed in *Volvox*, *Ciona*, and *Mya*; if they are associated with reversible photochemical reactions in the latter, one would expect them to be associated with similar reactions in the former. However, the response to light in the cercariae is also similar to the responses to light observed in *Amoeba*; and Folger (1925) contends that in *Amoeba* there is considerable evidence which indicates that photic stimulation is not photochemical. He maintains that mechanical shock produces cessation in streaming just as light does, and that, after a reaction to a mechanical shock, a period of recovery must be allowed before a specimen will respond to light again. He

concludes that "this seems to indicate that the processes involved in mechanical and photic stimulation are fundamentally the same . . . , and it is evident, since mechanical stimulation is not photochemical, that photic stimulation cannot be photochemical."

In so far as can be ascertained, response in cercariae to mechanical and electrical stimulation is identical with that induced by increase in illumination. Therefore, if Folger's contentions are correct for *Amoeba*, it may be assumed that photic responses in cercariae are not correlated with photochemical reactions. It is evident from the above discussion that the results obtained do not clearly show the nature of response in cercariae. Some of the evidence seems to support the contention that responses are correlated with a photochemical process, while other evidence seems to support the contention that they are not.

SUMMARY

1. The cercariae of *Bucephalus elegans* respond to rapid increase in light intensity. This response is regular enough to be used in quantitative measurements.

2. Changes to which the cercariae of *Bucephalus elegans* are subjected must be fairly rapid in order to induce a response, in other words, the reaction is dependent on the rate of change in the magnitude of the stimulating agent. The response is therefore a "shock reaction."

3. When photic reaction-time is plotted against luminous intensity, the resulting curve simulates a hyperbola. The reaction-time therefore varies inversely with the intensity.

4. Reaction-time is made up of two components, viz., the stimulation period and the latent period. Both of these components vary with the intensity; the greater the intensity the shorter the stimulation period and the shorter the latent period. The maximum stimulation period and the maximum latent period occur at the same intensity, i.e., at the lowest intensity.

5. Stimulating efficiency varies with the luminous intensity; as the intensity increases, the amount of light energy necessary to induce a response gradually increases to a maximum, then gradually decreases to a minimum. This indicates that the Bunsen-Roscoe law

does not hold for cercariae under the conditions of the experiments made.

6. Some of the evidence presented seems to indicate that response to light in the cercariae of *Bucephalus elegans* is associated with a photochemical reaction. Other evidence lends support to the contention that it is not photochemical.

7. The effect of temperature on photic reaction-time is such that the reaction-time varies inversely with the temperature.

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THE RELATION BETWEEN TEMPERATURE AND
FREQUENCY OF CONTRACTION IN THE
TAIL-FURCAE OF BUCEPHALUS
ELEGANS¹

(Four figures)

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THE relation between temperature and various vital processes has been investigated extensively both in plants and animals. It was early known that biological processes are, within certain limits, accelerated by raising the temperature and retarded by lowering it. It was not until recently, however, that any attempt was made to study the phenomena quantitatively with the view of ascertaining the nature and the significance of the reactions involved.

The relation between temperature and the rate of chemical reactions, in terms of the van't Hoff rule is often expressed as follows:

$$\frac{k_0 \left(\frac{10}{t_0 - t_1} \right)}{k_1} = Q_{10}$$

in which K_1 and K_0 are the velocity constants at temperatures t_1 and t_0 , respectively, and Q_{10} the temperature coefficient for 10° intervals. Simply stated, this rule means that for every change of 10° the velocity of a reaction increases or decreases a certain number of times. For most chemical reactions the value of Q_{10} lies between 2 and 3.

Kanitz (1905) showed that increase in temperature results in increase in carbon dioxide assimilation in plants and that over a wide range of temperatures the value of Q_{10} lies between 2 and 3. Blackman (1905), in reference to the same phenomenon in plants, and

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Snyder (1905), in reference to the frequency of contraction in isolated terrapin heart, came to the same conclusion.

In an extensive review of the literature Kanitz (1915) maintains that his previous contention holds for a wide variety of phenomena, the most striking of which follow: speed of nervous impulse, frequency of pulsation of the contractile vacuole, rhythmical contraction of smooth muscle, respiration in mammals, response to light and gravity in plants, protoplasmic streaming, permeability, and developmental processes.

Several other investigators have since studied the effect of temperature on vital processes. Crozier (1924*a*, *b*) and 1926*a*), Crozier and Federighi (1925*a* and *b*), and Crozier and Stier (1925) in an extensive study of the effect of temperature on various vital processes have attempted by means of the Arrhenius equation to identify the reactions associated with these processes at different temperatures. The relation between these reactions and temperature is given by the Arrhenius equation as follows:

$$\ln \frac{k_2}{k_1} = \frac{\mu}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right)$$

in which K_1 and K_2 equal the velocity constant at the two absolute temperatures T_1 and T_2 , R is the gas constant for a given chemical reaction. They calculated the value of μ in this equation and they assume that in so doing they ascertained the energy necessary to change a molecule from the inactive to the active state. They maintain that their results show several "critical temperatures" each characterized by an abrupt change in the value of μ , and that the values of μ found in vital processes are similar to those found in specific catenary reactions, and they consequently conclude that in organisms there are, associated with responses to temperature, catenary reactions of the same kind.

Heilbrunn (1925) maintains that protoplasm does not behave as a homogeneous system and that the fact that Crozier does not take into account the effect of temperature on viscosity, coagulation permeability, and the like invalidates the interpretation of the meaning of changes in the value of μ in the Arrhenius equation.

Belehradek (1930) contends that critical temperatures probably do not occur in organisms, that many vital processes do not follow any given rule precisely, and that the Arrhenius μ is of no greater significance than the van't Hoff coefficient.

Krogh (1914), in a series of studies on the relation between temperature and the velocity of embryonic development of such forms as amphibia, fishes, insects, and echinoderms, maintains that in none of these forms can the relation be expressed even approximately by the van't Hoff formula but in all of these forms the relation is algebraic.

There is consequently considerable difference of opinion concerning the interpretation of the results obtained in observations on the effect of temperature on vital processes. It seems therefore highly desirable to extend these observations. For this reason a study of the effect of temperature on cercariae was made. Moreover, such a study is also of interest owing to the fact that nothing is known concerning the response to temperature in these organisms.

MATERIAL AND METHODS

The method of obtaining cercariae and the apparatus used to regulate temperature have already been described (Bevelander, 1933). Instead of using an observation aquarium, several pieces of glass tubing sealed at one end and bent so as to resemble a boot were used to confine the cercariae to a limited region while observations were made.

Observations on the frequency of contraction of the tail-furcae were made as follows: An actively swimming specimen was put into a glass boot together with about 1 cc. of water. Then one end of a short piece of a small rubber tube was slipped over the open end and a thermometer was inserted through the rubber tube into the glass boot. In this manner the bulb of the thermometer was firmly held in place only a few millimeters from the specimen under observation. The boot was now put into a water bath at room temperature (18°) and left at least fifteen minutes, after which the frequency of contraction in the tail-furcae was observed for one minute and recorded. The water in the water bath was then replaced with water from a reservoir approximately 3° lower than room temperature. This tem-

perature was maintained for fifteen minutes after which the frequency of contraction was again ascertained in the same way. This process was then successively repeated, the temperature being lowered approximately 3° each time, until the tail-furcae no longer

TABLE I

RELATION BETWEEN TEMPERATURE AND FREQUENCY OF CONTRACTION IN THE TAIL-FURCAE OF CERCARIAE

Interval between observations at different temperatures fifteen minutes. Observations were made in the order shown in the tables. Column headed $\frac{1}{T}$, reciprocal of absolute temperature; Log. of Av., logarithm of the average frequency; no, reading not taken.

TEMPERATURE IN DEGREES C.	DESIGNATION OF SPECIMEN USED					AVERAGE	$\frac{1}{T}$	LOG. OF AV.
	1	2	3	4	5			
	Contractions per Minute							
	Individual Results							
18.....	52	48	no	48	48	49.0	0.00346	1.69
14.....	36	32	34	36	32	34.0	0.00348	1.53
10.....	20	20	22	22	20	20.8	0.00353	1.31
6.....	12	10	12	12	12	11.6	0.00354	1.06
3.....	5	2	0	3	5	3.0	0.00362	0.47
0.....	2	0	0	0	2	0.8	0.00367	0.03
- 2.....	0	0	0	0	0	0.0		
0.....	0	0	0	0	0	0.0		
5.....	7	6	0	7	6	5.2	0.00359	0.71
9.....	16	16	14	20	20	17.2	0.00354	1.23
14.....	33	32	36	36	34	34.2	0.00348	1.53
19.....	50	no	50	48	48	49.0	0.00338	1.70
22.....	64	64	68	66	no	65.5	0.00339	1.81
25.....	98	94	88	96	no	94.0	0.00335	1.97
28.....	100	98	104	106	no	102.0	0.00333	2.00
32.....	80	78	80	76	no	78.5	0.00326	1.89
38.....	12	14	15	20	no	15.2		
40.....	0	0	0	0	no	0.0		

showed any signs of movement. Then the temperature was raised approximately 3° and the process successively repeated until the temperature became so high that the tail-furcae no longer contracted. In this way the frequency of contraction was ascertained in each of 18 specimens in temperatures ranging from 0° to 40°. The results obtained are presented in Tables I and II and Figures 1 and 2.

FREQUENCY OF CONTRACTION IN THE TAIL-FURCAE

Examination of Table I, a protocol for five experiments, shows that, as the temperature decreased from 18° to 0° , the average frequency of contraction of the tail-furcae decreased from 49 to 0.8 per minute, and that, as the temperature increased from 0° to 40° , the frequency increased from 0 to a maximum of 102 at 28° , after which it rapidly decreased to 0 at 40° . This change in frequency is typical for all the experiments performed. It is clear, therefore, that temperature has a very decided effect on the frequency of contraction in the tail-furcae of cercariae.

Figure 1 shows more clearly the relation between temperature and the frequency of contraction in the tail-furcae. The curve in this figure is sigmoid in nature, similar to those so frequently obtained for biological reactions.

Table II which contains a summary of the results obtained with 18 specimens shows that as the temperature increased from 3° to 40° the van't Hoff temperature coefficient decreased from 29.8 to 0.0. This indicates that the processes associated with contraction vary continuously with the temperature.

It has been maintained that, when the logarithm of the rate or the frequency observed in biological phenomena is plotted against the reciprocal of the absolute temperature and a straight line results, the value of the Arrhenius μ is constant and the processes involved in the phenomena are constant, and that, when more than one straight line occurs, the value of μ is not constant, and there are critical temperatures at the points at which the lines cross.

The logarithm of the average frequency of contraction of 18 cercariae in different temperatures was plotted against the reciprocal of the absolute temperature and the results obtained are presented in Figure 2. This figure shows that the curve thus produced is not a straight line or a series of straight lines. It consequently indicates that the value of μ and the processes involved vary with the temperature.

ADAPTATION AND RECOVERY

An actively swimming cercaria was put into a glass boot, then a thermometer was inserted, after which the boot was put into the

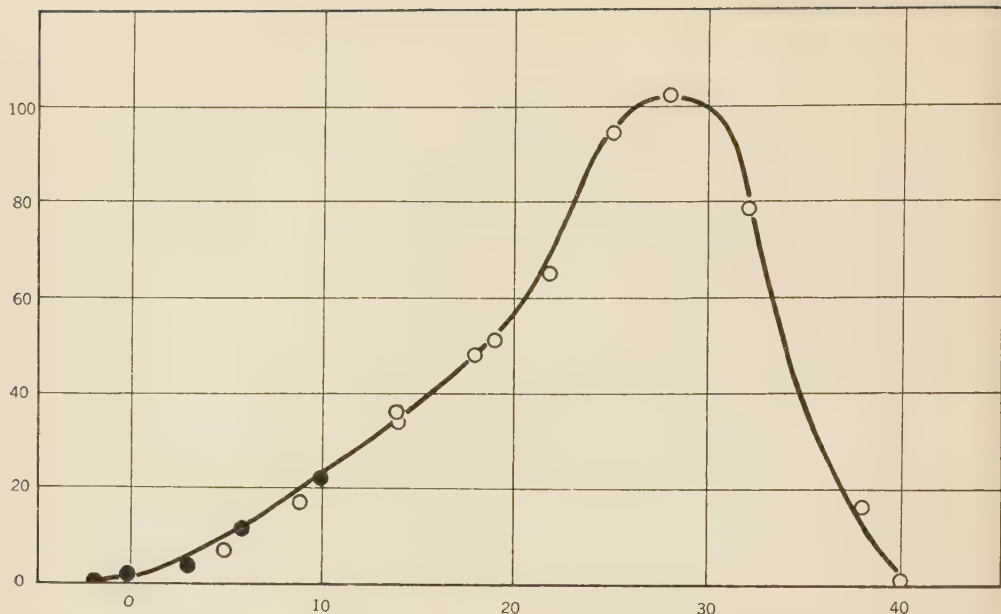


FIG. 1.—Relation between temperature and frequency of contraction. Ordinates, frequency; abscissae, temperature; dots, frequency after lowering the temperature; circles, frequency after raising it. Each point on the curve is an average of five specimens.

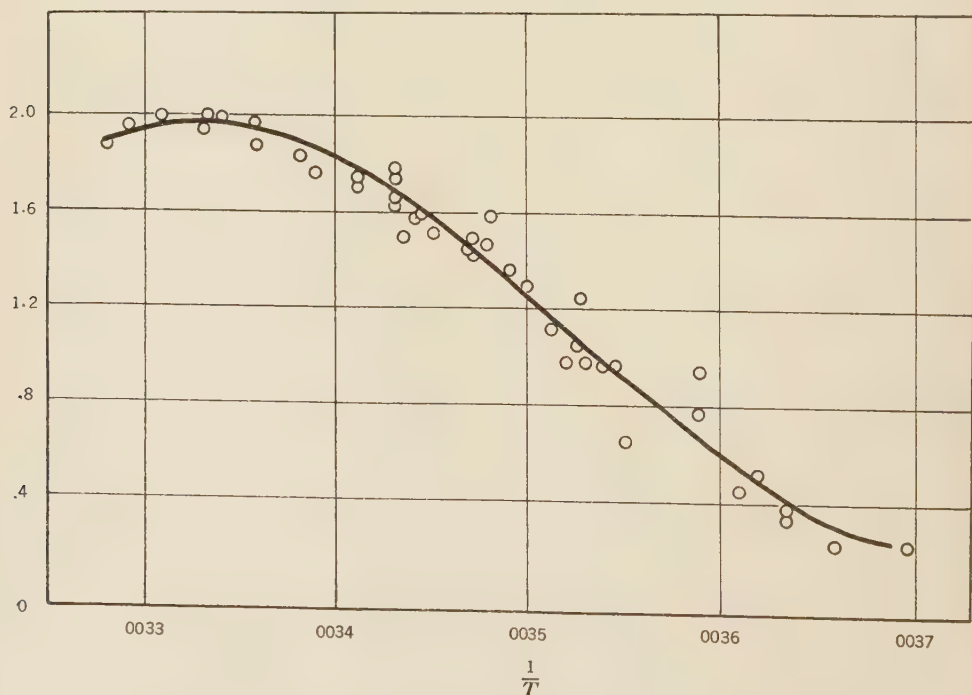


FIG. 2.—Curve showing the relation between temperature and frequency of contraction. Ordinates, logarithm of frequency; abscissae, reciprocal of absolute temperature. Each point on the curve represents an average of five readings made on eighteen specimens.

water bath at room temperature, left fifteen minutes, and the number of contractions in the tail-furcae in one minute ascertained. Immediately following this, the temperature was decreased to 4°, held for one minute, and then raised to room temperature. The frequency of contraction was now lower than it had been before the temperature was lowered. The time required to regain the former frequency was now ascertained; then the temperature was again decreased to 4°, held two minutes, and then raised to room temperature, after

TABLE II
AVERAGE RESULTS OF EIGHTEEN SPECIMENS
SHOWING RELATION BETWEEN TEMPERATURE,
AVERAGE FREQUENCY, AND TEMPERATURE CO-
EFFICIENT

Temperature (Degrees)	Average Frequency	Q ₁₀
3.....	0	
5.....	2.2	
10.....	12.0	29.75
15.....	31.8	7.02
17.....	38.0	2.43
19.....	50.1	3.98
23.....	78.0	3.02
26.....	87.8	1.48
28.....	100.0	1.92
31.....	89.6	0.69
36.....	43.6	0.24
38.....	13.5	0.00

which the time required to regain the original frequency was again ascertained. This was successively repeated leaving the cercaria longer and longer in the low temperature until an interval of ten minutes was reached. Then the whole process was repeated with 4 other specimens. It was now repeated with each of 6 different specimens subjected to different temperatures for the same length of time in place of the same temperature for different lengths of time. The results obtained are presented in Tables III and IV and Figures 3 and 4.

Table III shows that the longer the cercariae were left in the low temperatures, the longer they required to recover, the time for recovery being 1 min. 43 sec. if left one minute at 4°, and 9 min. 49

TABLE III
RELATION BETWEEN EXPOSURE TO 4° FOR VARYING INTERVALS AND THE
TIME REQUIRED FOR RECOVERY

EXPOSURE TIME IN MINUTES AT 4°	DESIGNATION OF SPECIMENS					AVERAGE
	1	2	3	4	5	
	Recovery Time in Minutes					
	Individual Results					
1.....	1'30"	1'40"	1'48"	1'56"	1'42"	1'43"
2.....	2'10"	2'35"	2'21"	2'18"	2'5"	2'18"
3.....	2'48"	2'55"	2'50"	2'30"	2'58"	2'48"
4.....	3'52"	4'15"	4'18"	4'25"	3'45"	4'7"
5.....	4'42"	4'30"	4'50"	5'5"	5'	4'49"
6.....	6'	6'12"	6'10"	6'20"	6'24"	6'13"
8.....	7'44"	8'	7'25"	7'35"	7'52"	7'43"
10.....	9'30"	10'24"	9'25"	9'5"	9'52"	9'39"

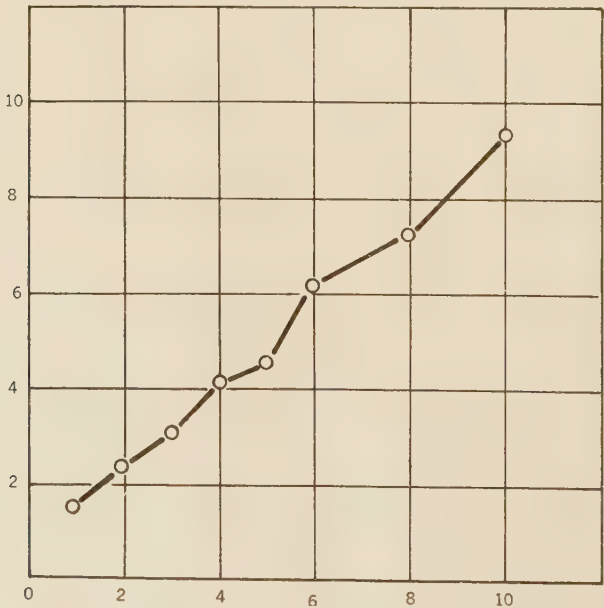


FIG. 3.—Relation between exposure of cercariae at 4° for different intervals, and the time required to recover at room temperature (17°).

sec. if left 10 minutes. This indicates that recovery time is nearly equal to the exposure period. This is more clearly shown in Figure 3.

Table IV shows that as the temperature at which the specimens were left ten minutes decreased or increased from room temperature, the time required to recover at room temperature increased. It also shows that the time required to recover after exposure at 5° is nearly

TABLE IV

RELATION BETWEEN EXPOSURE FOR TEN MINUTES AT VARIOUS TEMPERATURES, AND THE TIME REQUIRED TO RECOVER AT ROOM TEMPERATURE

EXPOSURE TEM- PERATURE (DEGREES)	DESIGNATION OF SPECIMENS						AVER- AGE
	1	2	3	4	5	6	
	Recovery Time in Minutes						
	Individual Results						
5.....	6'50"	6'07"	6'22"	6'46"	6'00"	6'25"	6'25"
9.....	3'30"	3'20"	3'25"	3'40"	3'15"	3'25"	3'25"
12.....	1'55"	2'15"	2'05"	2'12"	2'02"	2'05"	2'05"
17.....							
20.....	1'40"	1'20"	1'27"	1'36"	1'41"	1'30"	1'32"
25.....	2'05"	2'15"	2'00"	2'08"	2'18"	2'10"	2'09"
30.....	4'00"	4'09"	4'17"	4'05"	4'10"	4'09"	4'08"
35.....	4'45"	4'35"	4'18"	4'33"	4'40"	4'35"	4'34"

six times as great as it is after exposure at 12° , and that after exposure to 35° it is nearly four times as great as it is after exposure to 20° . Figure 4 shows this relation even more clearly. It shows that from 5° to 17° the time for recovery is inversely proportional, and that from 17° to 35° it is directly proportional to increase in temperature.

One noticeable difference between adaptation at high and low temperatures in cercariae is the effect on reversibility in recovery. Specimens usually recover from exposure to low temperature (0° , e.g.) without any noticeable effect, that is, reversibility is complete. At high temperatures, however, this does not obtain.

DISCUSSION

As previously stated, the effect of temperature on biological processes has been studied by many investigators who seem to differ in their interpretation of the phenomena. Some investigators maintain that their data are accurately expressed by the van't Hoff equation; others employ the Arrhenius formula; special equations either algebraic or exponential are also used to some extent. Another group

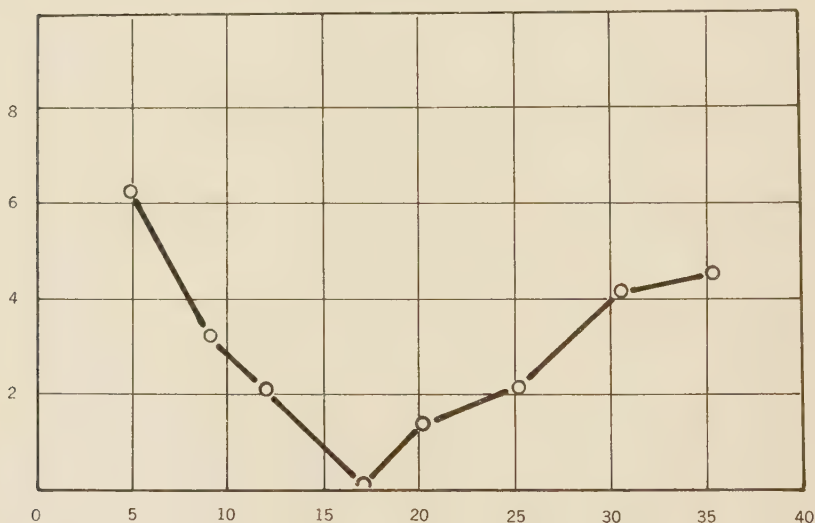


FIG. 4.—Relation between temperature to which cercariae had been subjected for ten minutes, and recovery time at room temperature (17°).

holds that in dealing with a heterogeneous system such as protoplasm that temperature affects several processes with different velocities and accordingly the meaning of temperature coefficients is of little value. If this latter idea is correct, then the data do not represent the effect of temperature upon any one process, but on several processes which occur simultaneously.

Temperature changes in cercariae undoubtedly affect several physico-chemical changes in the organism at different velocities. It seems quite probable that the longitudinal and circular muscles in the furcae would react with different velocities and that changes in viscosity, coagulation, permeability, and the like would also be

affected differently at different temperatures. Thus, it does not seem at all unreasonable to find that the data presented for cercariae do not lend themselves very well to formulation over a wide range of temperatures. It is of interest to note, however, that, when the data are represented graphically, a sigmoid curve results, as is so frequently the case in experiments of this kind.

SUMMARY

1. Temperature affects the frequency of contraction of the tail-furcae very markedly. As the temperature increases from 0° , the frequency of contraction increases from 0 to a maximum of 102 at 28° , after which it rapidly decreases to 0 at 40° .

2. The temperature coefficient decreases from 29.8 at 3° to zero at 38° . This indicates that the processes associated with change in temperature vary throughout the whole range investigated. The evidence at hand does not indicate the occurrence of "critical temperatures."

3. If the temperature is decreased and held for a given length of time and then increased again, the frequency of contraction in the tail-furcae is much lower than it was and the time required for recovery depends specifically upon the extent of the decrease in temperature and the time the cercariae are exposed to it.

4. If specimens are exposed to temperatures between 1° and 4° the frequency is completely reversible. If they are exposed at 40° the frequency is incompletely reversible or not reversible at all, depending upon the time of exposure.

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VITA

Gerrit Bevelander was born in West Sayville, New York, April 6, 1905. He attended the public schools of Sayville, being graduated from High School in 1922. The following year he entered Hope College and received the degree of Bachelor of Arts in 1926. In 1927 he attended the University of Michigan where he pursued studies in the Zoölogical sciences, receiving the degree of Master of Arts in June, 1928. The following year he was appointed graduate assistant in the department of Zoölogy at The Johns Hopkins University. During the next three years the requirements were met and completed for the degree of Doctor of Philosophy. The summers of 1929 and 1930 were spent as field investigator for the U.S. Bureau of Fisheries and that of 1931 at the Mt. Desert Island Biological Laboratory as independent investigator.

